TRANSLATION NO. 993

DATE: 30 Dec. 1963

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

[Following is translation of an article by R. Haas, R. Thomssen and H. Roth in the German-language Journal Deutsche Medizinische Wochenschrift (German Medical Weekly), Vol. 86, 1961, No. 45, pp. 2141-2144.]

RAPID ACTIVE IMMUNIZATION AGAINST TETANUS

According to many authors (1, 2, 3), simultaneous inoculation represents today, in cases of injury, for nonimmunized or insufficiently immunized persons the method of choice for tetanus prophylaxis. It consists as a rule in the more or less simultaneous but locally separated injection of at least 1,500 international units of tetanus antitoxin and of a dose of tetanus vaccine. Active tetanus immunization initiated with the first injection of tetanus vaccine is usually complemented by the injection of a second dose of vaccine 4-6 weeks later. The second dose of vaccine must not be omitted and can, if absolutely necessary, at most be somewhat retarded.

The purpose of simultaneous immunization is evident. The administration of antitoxin provides the patient in the shortest interval of time with a certain concentration of antitoxin. However, the latter begins to drop immediately after creation in accordance with an exponential law. To this is due the short duration of this passive protection. The simultaneously induced active immunization has the purpose of replacing the receding passive immunity as fast as possible by active immunity. Its advantages become effective primarily only for eventual later injuries because we can forego an injection of antitoxin and therefore avoid the risks in connection with the injection of animal sera (anaphylactic shock, allergic shock, serum disease). If the simultaneous inoculation was tried out as prescribed, i. e. if it consisted of at least two injections of vaccine in addition to the injection of antitoxin, in a possible later new injury or other exposure depending on the point in time of its occurrence, either no specific prophylactic measures are effected or a dose of vaccine is given (booster injection). Anyone who decides to administer in principle the vaccine in a new injury, cannot commit an error.

It is occasionally regarded as a certain disadvantage of active immunization that a relatively long interval seems to elapse until it leads to a concentration of antitoxin regarded as of sufficient

stability. As a rule, this is the case only a few days after the second injection of vaccine. If healthy individuals are immunized, this disadvantage is of no significance. The case is different in simultaneous inoculation. It is here desired obviously to have a method of active immunization which leads so quickly to the formation of tetanus antitoxin that it will still benefit the late tetanus case. There is hardly any doubt that the present form of simultaneous prophylaxis is not able to accomplish this. However, no facts are known which would have to be interpreted in another sense. Perhaps it is impossible in principle to convert a receding heterologous passive immunity into active immunity without any gap. However this may be, the situation has induced us to investigate if it is possible, by changing the customary intervals of inoculation with or without increase of the injections, to obtain adequate antitoxin concentration in the blood earlier than otherwise. The following reports on the results of the findings.

Material and Methods

Members (males) of the German Red Cross in Freiburg/Br. volunteered for the investigation. Two groups of 16-17 each individuals were formed for whom a basic immunization with tetanus vaccine had neither begun or terminated. None of the individuals had suffered from tetanus. Some of the individuals had already been immunized passively once or several times. The last administration of serum then was always more than 4-5 years in the past. The distribution of age was as follows: 13 individuals from 14-20 years; 6 individuals from 21-25 years; 5 individuals from 26-33 years; and 9 individuals from 34-54 years.

For immunization, we utilized the aluminum hydroxide adsorbate toxoid "tetanol" of the Behring plants, Marburg/L. The vaccine had the control No. 44 and had been tested in the Paul-Ehrlich Institute on 17 October 1957. 0.5 ml of the vaccine contained not less than 75 IU. Innoculation began on 10 July 1958.

The vaccine was injected subcutaneously and alternately in the subdeltoid region of both arms and, for some individuals, also in the infraclavicular region if the infiltrations in the upper arm were too large.

Group I comprised 16 individuals, each of which received 2 toxoid injections at an interval of 10 days. 1.0 ml were injected on the first and 0.5 ml "tetanol" were injected on the tenth day.

Group II comprised 17 individuals, each of which received 5 toxoid injections at intervals of 2 days each. Injections consisted

of 1.0 ml on the first and 0.5 ml of "tetanol" on the third, fifth, seventh and ninth day.

Evaluation was effected through serum antitoxin determination in mice. In addition to the blood specimen prior to vaccination, such specimens were taken on the 13th, 19th, 39th, 69th and 129th day after start of vaccination and, in Group II, additionally on the ninth day after the start of vaccination. Determination of antitoxin titer was carried out in the customary manner by employing test toxin and standard antitoxin. All indications of titer are in international units per ml.

Findings

Table 1 and 2 contain the data on antibody concentration measured in the sera of the individuals obtained at different times subsequent to vaccination. Group I reached a mean antibody titer of 1.84×10^{-3} IU/ml 20 days after begin of vaccination and after subsequent steep rise, a titer of about 2.5×10^{-2} IU/ml on the 39th day. As long as examination took place, no further essential change occured during the following four months.

Table 1. Antibody titer individuals injected with "tetanol" in Group I, in IU/ml serum

a Name	b Alter	c vor Immunisierung	d 9. Tag	e 13. Tag	f 19. Tag	g 39. Tag	h 69. Tag	i 129. Tag
V.H.	16	0,001	—	0,001	0,02	0,05	1,0	2,0
K.R.	17	0,001	—	0,001	0,001	0,05	0,1	0,05
Z.A.	18	0,001	—	0,001	0,005	—	—	0,02
K.K.	18	0,001	—	0,001	0,001	0,005	0,05	0,02
W.R.	21	0,001	—	0,001	0,001	—	0,02	0,02
B.H.	21	0,001	—	0,001	0,001	0,005	—	0,1
D.G.	22	0,001	—	0,001	0,001	0,1	0,05	—
Z.E.	25	0,001	—	0,001	0,001	0,005	—	—
F.A.	29	0,001	—	0,001	0,001	—	0,01	0,02
Z.A.	30	0,001	—	0,001	0,001	0,005	0,005	0,01
K.B.	31	0,001	—	0,001	0,01	0,2	0,05	0,01
D.E.	33	0,001	—	0,001	0,001	0,002	0,005	0,01
N.H.	34	0,001	—	0,001	0,005	0,05	0,02	0,02
H.E.	38	0,001	—	0,001	0,05	0,1	0,05	0,05
T.M.	43	0,001	—	0,001	0,001	0,01	0,01	0,01
D.E.	54	0,001	—	0,001	0,001	0,005	0,002	0,01
k Geometr. Mittelwerte:		$1,0 \times 10^{-3}$		$1,0 \times 10^{-3}$	$1,84 \times 10^{-3}$	$2,5 \times 10^{-2}$	$2,5 \times 10^{-2}$	$2,5 \times 10^{-2}$

a — name; b — age; c — before immunization; d-i — 9th, etc day; k — geometrical mean values.

Table 2. Antibody titer of individuals in Group II injected with "tetanol", in IU/ml serum

Name	Age	Before immunization	9th day	13th day	19th day	39th day	69th day	129th day
K.P.	14	0.001	0.001	0.001	0.05	0.1	0.5	0.5
J.U.	15	0.001	0.001	0.001	—	0.002	0.01	0.01
V.P.	16	0.001	0.001	0.005	2.0	1.0	1.0	0.2
R.K.	16	0.001	0.001	0.001	0.1	2.0	—	0.5
E.G.	16	0.001	0.001	0.001	0.1	0.5	0.2	0.2
E.W.	16	0.001	0.001	0.001	0.05	—	0.5	0.5
G.F.	16	0.001	0.001	0.001	0.05	0.5	0.5	0.2
S.H.	16	0.001	0.001	0.001	0.02	0.5	0.5	0.5
K.P.	17	0.001	0.001	0.001	0.1	—	0.5	0.2
W.H.	21	0.001	0.001	0.001	0.02	0.2	0.1	0.1
D.U.	23	0.001	0.001	0.001	0.05	0.2	0.2	0.2
Z.H.	28	0.001	0.001	0.001	0.05	0.2	0.5	0.2
J.K.	34	0.001	0.001	0.001	0.005	0.1	—	—
E.H.	37	0.001	0.001	0.001	0.001	0.01	0.2	0.1
F.W.	39	0.001	0.001	0.01	0.1	1.0	1.0	—
E.J.	42	0.001	0.001	0.001	0.001	0.1	—	0.1
N.W.	48	0.001	0.001	0.001	0.002	0.01	0.01	—
geometrical mean values		1.0×10^{-3}	1.0×10^{-3}	1.28×10^{-3}	2.84×10^{-2}	1.48×10^{-1}	2.55×10^{-1}	2.08×10^{-1}

The cause of antitoxin formation in Group II was similar but the steep rise set in earlier and reached prior to the 20th day a value above the mean value of Group I and rose further to 7-8 times of the value of Group I, i.e. $1.5-2.0 \times 10^{-1}$ IU/ml.

Another picture of the differing effect of the two methods of immunisation is offered by the consideration of the individual titer curves within the two groups. In Group I, the individuals reacted individually rather differently in regard to the speed and extent of antibody formation. In Group II, this behavior gives way to a more uniform type of reaction with a much smaller individual spread.

If we assume a concentration of 0.005 IU/ml antitoxin as the lowest value affording protection against an intoxication as it may occur in man as the consequence of an injury, then the bulk of the individuals of Group II reached this limit already prior to the 20th day after the start of immunisation, i.e. 13 out of 17 individuals. In Group I, only 4 of 16 individuals reached this value prior to the 20th day, 9 out of 16 only after 30 days. The relations are grouped in Table 3.

The individual values were obtained by interpolation. We obtained, as average elapsed interval for exceeding 0.005 IU/ml, 35.5 days in Group I and 20.5 days in Group II. With a mean quadratic deviation of $s_1 = 15.35$ and $s_2 = 7.30$ and the degrees of freedom $n_1 = 15$ and $n_2 = 16$, we obtain a difference of the mean values which

cannot be regarded as random (t = 3.6 at a $P_{0.001}$ of 3.63). The difference in the variations of the individual value can also be determined exactly and can no longer be regarded as random:

$$F = \frac{s_1^2}{s_2^2} = 4.44 \text{ and } P_{0.01} = 3.46$$

Table 3. Distribution of intervals after which individual titers reached or exceeded 0.005 IU/ml

Tage nach Beginn der Impfungen a	10–14	15–20	20–25	25–30	over 30
Gruppe I bei 0.005 I.E/ml b	—	17, 17, 18, 20	—	27, 27, 28	34, 42, 42, 42, 42, 42, 45, 55, 70
Gruppe II bei 0.005 I.E/ml c	12, 14	15, 15, 15, 16, 16, 16, 16, 16, 17, 17, 20	—	26, 29	32, 35

a -- days after start of vaccination; b — Group I at 0.005 IU/ml; c -- Group II at 0.05 IU/ml

As findings, we can now indicate that only 19% of Group I had reached the critical limit of 0.005 IU/ml on the 19th day after start of immunization whereas 80% of Group II were already protected at this point. Moreover, the critical limit is reached more uniformly in Group II.

Throughout the entire experimental series, no complications due to vaccination occurred in any individual which would have reduced the capability for work. Generally, only local reddening occurred at the point of vaccination which exceeded the diameter of 4–5 cm only in infrequently cases and sometimes changed into somewhat larger infiltrates. Four months after start of vaccination, the points of vaccination no longer showed any observable signs.

Discussion

A so-called basic or foundation immunization against tetanus consists of two injections of vaccine which are generally administered at an interval of at least 4–6 weeks. If this interval is observed, it may be anticipated that, a few days after the second injection, a high percentage of a group regarded as representative will have formed tetanus antitoxin in an amount considered as sufficient.

Few reliable indications can be found in literature of the

condition under which shorter intervals between two injections are sufficient in order to achieve the desired effect. It would here be necessary first to define what is meant by desired effect. It is conceivable that this effect may be considered as reached in some cases when, at a shortened interval of the two injections, the antitoxin concentration considered as adequate may perhaps not be determined one week after the second injection but perhaps two, three or four weeks later. In other words, there are situations in which it is relatively unimportant when the antitoxin threshold considered as necessary is exceeded and where we are only concerned that this be the case. For example, this can be the case if healthy individuals are available only for a relatively short time for carrying out the vaccination. On the other hand, situations may be imagined, as is shown by the example of simultaneous vaccination in the case of injury, in which it is important not only to complete active immunization itself in a short time but to exceed moreover the critical antitoxin threshold of the blood as far as possible. Another question in this connection is the one whether, in the case of non-immunized and injured patients, we can permit the administration of antitoxin as such and attempt to achieve the desired result instead by giving toxoid injections.

In order to obtain some information on the questions outlined, we carried out active tetanus immunization on two groups of individuals. A series of practical viewpoints were decisive for the experimental arrangement. We utilized tetanus adsorbate vaccine for the first and second injections, although this procedure is not necessarily to be regarded as optimum from a theoretical standpoint. D'Antona and Piazzi (4) have shown that it is possible to obtain much higher antitoxin titer if only the first vaccination is performed with adsorbate vaccine and the later injections with liquid toxoid free of adjuvants. This corresponds to the general rules of immunization with vaccines containing adjuvants (8). However, we decided on the procedure prescribed in spite of this because today only adsorbate toxoids are available commercially in the Federal Republic and the practicing physician is restricted to these preparations.

Since only relatively few voluntary individuals were available for the experiment, we have restricted the extent of the investigation somewhat. In place of a larger number of groups of individuals where it would have been possible to vary the interval between the two injections more greatly, we have formed, in view of the relative total number, only two groups and operated merely with a uniform shortening of the interval to 10 days. This interval was selected in order to test whether it would be possible to achieve immunization in an interval of time which would still be in a reasonable relation to the incubation time of tetanus. Rose (5) recorded among his material only

15% of cases of tetanus where the period of incubation was shorter than 10 days.

Since we anticipated that this interval would possibly be insufficient in order to accelerate the result of vaccination, we combined, in the second vaccinating plan, the shortening of the interval of vaccination to 10 days with an increase of the number of injections and consequently also of the total antigen dose.

The result of the investigations in Group I showed that the shortening of the interval as against the customary interval of 4-6 weeks does not accelerate the result of vaccination. The critical antibody concentration is reached by most of the individuals tested only after four weeks. However, there was available, on the 39th day, an adequately high, even though not entirely satisfactory antibody titer which testifies that the basic immunization was successful in spite of the short interval. We may prudently consider, in view of the small number of individuals, that in urgent cases, e.g. in the case of early discharge of a patient, the second dose can follow 10 days after start of immunisation with an expectation of success. However, it should be considered that the first injection in our experiments constituted double the customary dose.

Increase of the number of injections and therefore also of the total antigen in the second vaccination plan led to another result. On the 20th day after start of immunisation, over 80% of the individuals reached an antitoxin concentration in the serum at which probably affords protection against tetanus. Whether the possibility of actively accelerating the formation of tetanus antitoxin has significance in practice, primarily in cases of injury, we are unable to say. There is no doubt that it is possible, in the manner described, to create a certain antitoxin concentration in the blood withing a considerable percentage of the periods of incubation of tetanus. 10-25% of all tetanus cases are alleged to have periods of incubation of more than 20 days (5, 6). However, there is still the question whether the period of incubation represents the one factor which has decisive importance for the creation of active tetanus immunity in the race with the outbreak of the clinical sickness. Perhaps entirely different pathogenetic factors are more important. Furthermore, we need to clarify, for the case of simultaneous vaccination under the conditions described, how such a forced active immunisation affects passive immunity. Eckmann (1) did demonstrate that the acceleration of the elimination of antitoxin through the simultaneously administered toxoid can be neglected in the customary simultaneous prophylaxis. However, other investigations will have to examine the influence of the utilisation of higher amounts of toxoid on the elimination of passively developed antitoxin. However, it would appear certain in

any event that it will not be possible even with the forced active immunization method utilized by us to convert the receding passive antitoxic immunity into an active immunity without any gap. Finally, the possibility voiced of foregoing serum injection entirely because high toxoid injections can lead sufficiently quickly to an actively formed antibody concentration, finds no support in the present investigations. It is entirely possible to discuss the action and the value of possibly induced heterologous antitoxins from various viewpoints but to replace them by a toxoid injection on the basis of the present investigations would be tantamount to abandoning the basis of confirmed facts.

Summary

Two groups of 16-17 persons each were actively immunized against tetanus. The first group received two injections of 1.0 and/or 0.5 ccm at an interval of 10 days. The second group received a total of 5 injections of 1.0 ccm initially and 0.5 ccm subsequently at intervals of 2 days each. The vaccine utilized was "tetanol", control No. 44, of the Behring plants. The vaccine contained not less than 150 international units per ccm. Prior and up to the 129th day after start of vaccination, repeated determination of blood antitoxin was carried out six times in Group I and seven times in Group II. 0.005 IE/ccm serum are regarded as critical threshold value of the antitoxin concentration in the blood. The individuals of the first group reached and/or exceeded this value prior to the 29th day after start of immunization. Only 5 of 16 individuals of this group had reached and/or exceeded this value on the 19th day. 13 of the 17 individuals of Group II had reached or exceeded this value by the 19th day. The average antitoxin content in the blood of individuals of Group II was at least 15 times as high on the 19th day as that of the individuals of Group I and about 6 times as high on the 39th day.

References

(1) Eckmann, L. : Schweiz. med. Wschr. 89 (1959), 311.

(2) Eckmann, L. : "Tetanus: Prophylaxe und Therapie" (Basel-Stuttgart 1960)

(3) Regamey, R. : Ergebn. Mikrobiol. 32 (1959), 270.

(4) D'Antona, D., S. Piazzi: Rev. Immunol. (Paris) 20 (1956), 317.

(5) Rose, S. : Dtsch. Chirurgie Lfg. 8 (1097).

(6) Hübner, A., K. Freundenberg: Chirurg 25 (1954), 145.

(7) Haas, R., R. Thomssen: Ergebn. Mikrobiol. 34 (1961) 27.